

Amendments to the Claims

Please cancel Claims 1 – 13.

Please add new Claims 14 - 26 as shown below. This listing of claims will replace all prior versions and listings of claims in the application. The new Claims add no new matter to the Specification.

Listing of Claims

1. (Cancelled) A method for altering the offspring sex ratio of an animal, the method comprising the steps of:
 - a) preparing a transgene including in operable association (i) at least one post-meiotic spermatogenesis-specific expression regulatory sequence, wherein the regulatory sequence is selected from the group consisting of a Herpes Simplex Virus promoter (HSV) promoter, its mutated and truncated forms; (ii) a DNA sequence encoding a toxin whose expression interferes with sperm's ability to fertilize an oocyte, wherein the toxin is selected from the group consisting of thymidine kinase (tk), its mutated and truncated forms; (iii) an optional DNA sequence encoding a selectable marker; (iv) an optional loxP site-flanked intervening DNA sequence inserted between the sequences encoding the post-meiotic spermatogenesis-specific promoter and the toxin, wherein said intervening sequence prevents transcription of the DNA sequence encoding the toxin unless said intervening sequence is removed by Cre recombinase; (v) an optional cellular localization signal sequence that restricts the ability of the mRNA and protein from the said transgene to randomly diffuse among the inter-connected haploid spermatids; and (vi) two optional flanking DNA sequences allowing said transgene to be inserted onto specific loci of a sex chromosome by homologous recombination;
 - b) creating transgenic animals using said transgene so that the transgene is inserted onto one of the two sex chromosomes;
 - c) mating the males of the said transgenic animals with females, the females containing Cre recombinase activity to activate the said transgene wherein the transgene includes the optional loxP site-flanked intervening DNA sequence of step a) (iii); and

- d) identifying at least one transgenic animal with desirable reproduction features, specifically, alteration of offspring's sex ration, and thereby altering the offspring sex ratio of said animal.
2. (Cancelled) The method according to Claim 1, wherein said animals include all mammals and –non-mammal organisms using X and Y chromosomes to determine sex.
 3. (Previously Cancelled)
 4. (Cancelled) The method according to Claim 1, wherein the alteration of offspring's sex ratio of said transgenic animals is from 50% to 100%.
 5. (Previously Cancelled)
 6. (Cancelled) The method according to Claim 1, wherein said optional DNA sequences allowing said transgene to be inserted onto specific loci of the sex chromosome target the transgene to the X chromosome, wherein the optional DNA sequences are Hprt locus-specific sequences.
 7. (Cancelled) The method according to Claim 1, wherein said optional DNA sequences allowing said transgene to be inserted onto specific loci of the sex chromosome target the transgene to the Y chromosome, wherein the optional DNA sequences are Tspy pseudogene-specific sequences.
 8. (Cancelled) The method according to Claim 1, wherein the post-meiotic spermatogenesis-specific expression regulatory sequence is replaced with an embryonically-expressed promoter, and the DNA sequence encoding a toxin whose expression interferers with sperm's ability to fertilize an oocyte is replaced by a DNA sequence encoding a toxin whose expression interferes with embryonic development or viability, the method further comprising the steps of inserting a transgene bearing said DNA sequences onto one of the two sex chromosomes prevents embryos with one

particular sex chromosome from developing into individuals, preparing a transgene which comprises in operable association of (a) at least one expression regulatory sequence which expresses in early stage embryos but not during spermatogenesis for XY organisms or oogenesis for ZW organisms; (b) a DNA sequence encoding a toxin selected from the group consisting of diphtheria toxin and ricin whose expression kills the embryo or blocks the normal development of embryos; (c) a loxP site-flanked intervening DNA sequences inserted between the promoter and the toxin gene, and the said intervening DNA sequences can prevent the toxin gene transcription unless it is removed by Cre recombinase; (d) an optional DNA sequence encoding a selectable marker selected from the group consisting of neomycin-, hygromycin- or puromycin-resistance gene, Hprt selection cassette, and diphtheria toxin gene; (e) two optional flanking DNA fragments allowing said transgene to be inserted onto specific loci of the sex chromosome by homologous recombination creating transgenic animals, and breeding of the transgenic animals with animals that contain a Cre recombinase transgene driven by spermatogenesis- or oogenesis-specific promoters.

12. (Cancelled) The method according to Claim 1 wherein the step of creating transgenic animals includes the steps of creating transgenic animals from techniques selected from the group consisting of: pronuclear microinjection, retroviral vector transfection, lipofection, and sperm incubation, and examining the transgene integration site by FISH for each transgenic founder to search for individuals with the transgene inserted onto the desired sex chromosome.
13. (Cancelled) The method according to Claim 1 wherein the selectable marker of step a) ii) is selected from the group consisting of a neomycin-resistance gene, a hygromycin-resistance gene, a puromycin resistance gene, a Hprt selection cassette and a diphtheria toxin gene.
14. (New) A transgene comprising, in operable combination, i) a nucleotide sequence encoding a Herpes Simplex Virus (HSV) promoter, ii) a nucleotide sequence encoding HSV-thymidine kinase (HSV-tk), iii) a nucleotide sequence encoding a selection marker,

iv) a nucleotide sequence encoding a transcription regulator located between the HSV promoter and the HSV-tk sequence, wherein the nucleotide sequence prevents the transcription of HSV-tk and wherein the sequence is located between two Lox P sites, v) a nucleotide sequence encoding a protamine promoter, vi) a nucleotide sequence encoding a cellular localization signal and, vii) two flanking sequences, wherein the two flanking sequences allow for the insertion of the transgene into a sex cell.

15. (New) The transgene of Claim 14, wherein said flanking regions are selected from a group consisting of TSPY flanking regions and HPRT flanking regions.
16. (New) The transgene of Claim 15, wherein said HPRT flanking regions are amplified via PCR using primers selected from a group consisting of SEQ ID NOs: 1 - 4 and said TSPY flanking regions are amplified via PCR using primers selected from a group consisting of SEQ ID NOs: 5 - 8.
17. (New) A method comprising the transfection of the transgene of Claim 14 into embryonic stem cells to create transfected embryonic stem cells.
18. (New) A method comprising the introduction of the transfected embryonic stem cells of Claim 17 into blastocyte embryos to create transfected blastocyte embryos.
18. (New) A method comprising the transfer of the transfected blastocyte embryos of Claim 18 into the uterus of one or more pseudopregnant females of the same species as the transfected blastocyte embryos and carrying the pregnancy to produce offspring.
19. (New) A method comprising the breeding of the offspring of the females of Claim 18 such that the female offspring comprising the transgene comprising the HPRT flanking regions are breed to wild type males to produce offspring.

20. (New) A method comprising the breeding of the offspring of the females of Claim 18 such that the male offspring comprising the transgene comprising the TSPY flanking regions are breed to wild type females to produce offspring.
21. (New) A method comprising screening the offspring of Claim 19 to identify offspring comprising the transgene.
22. (New) A method comprising screening the offspring of Claim 20 to identify the offspring comprising the transgene.
23. (New) A method comprising breeding the male offspring comprising the transgene of Claim 21 to wild type females to produce offspring.
24. (New) A method comprising breeding the male offspring comprising the transgene of Claim 22 to transgenic females comprising a transgene for Cre recombinase activity to produce offspring.
25. (New) A method comprising determining the gender of the offspring of Claim 23.
26. (New) A method comprising determining the gender of the offspring of Claim 24.